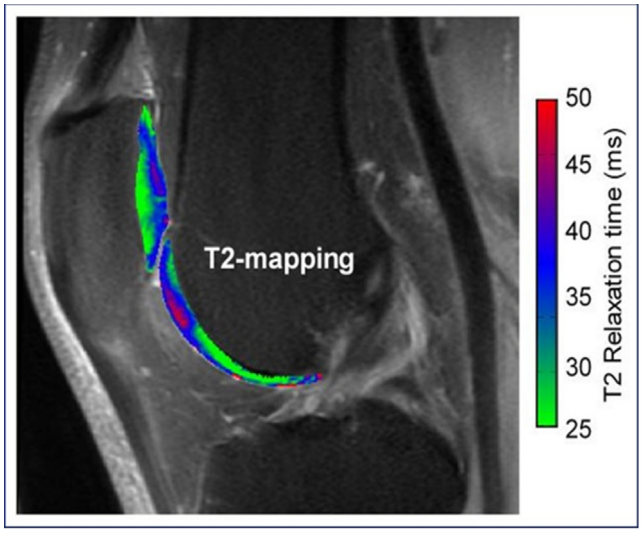
important role in the etiology of PFP. It has been suggested that a change in cartilage composition, due to deterioration of structural components like collagen and glycosaminoglycan's (GAGs), precedes morphological characteristics of cartilage damage in OA patients. With innovative MRI sequences, including T1ρ and T2 mapping, it is possible to measure these early changes in cartilage composition quantitatively by measuring cartilage content. Therefore, the purpose of this study was to investigate differences in cartilage composition between patients with PFP and control subjects using quantitative T1ρ and T2 mapping MRI.
Methods: Patients diagnosed with PFP and healthy control subjects aged 14-40 years were included in a cross-sectional case-control study. Measures included a questionnaire, physical examination and MRI at 3T. MRI comprised morphologic, T1ρ and T2 mapping sequences. T1ρ and T2 mapping sequences were conducted to measure cartilage glycosaminoglycan and collagen content, respectively. In-house developed software was used for image post-processing in order to calculate T1ρ and T2 relaxation times (see Figure 1). Higher relaxation times equal less content and less content equals a lower cartilage quality. Differences in T1ρ and T2 relaxation times for trochlear and patellar cartilage were compared between patients and control subjects by linear regression analyses, adjusted for potential confounders.
Results: 59 patients and 67 control subjects were included. BMI was significantly lower and sports participation significantly higher in control subjects. Mean T1ρ relaxation times of the patellar (46.8 vs 46.1 milliseconds (ms), $p = 0.94$) and trochlear cartilage (50.9 vs 50.1 ms, $p = 0.52$) did not significantly differ between patients and control subjects. In addition, no significant difference was seen between patients and control subjects in mean T2 relaxation times of patellar (33.4 vs 32.8 ms, $p = 0.16$) and trochlear cartilage (36.8 vs 36.6 ms, $p = 0.70$) (see Table 1).
Conclusions: Our findings suggest that cartilage composition, measured by T1ρ and T2 mapping, does not play a role in the etiology of PFP. However, follow-up research will demonstrate potential regional differences within the patellar and trochlear cartilage.

Table 1
T2 and T1ρ relaxation times (ms) of trochlear and patellar cartilage (Mean(sd)).

	Patients (N=59)	Controls (N=67)	Mean difference (+95% CI)	P-value
T2 trochlea	36.81 (2.55)	36.63 (2.39)	0.18 [-0.69, 1.05]	0.70a
T2 patella	33.41 (2.86)	32.82 (2.56)	0.59 [-0.36, 1.55]	0.16b
T1ρ trochlea	50.85 (3.57)	50.13 (4.03)	0.72 [-0.72, 2.17]	0.52b
T1ρ patella	46.79 (4.21)	46.09 (4.43)	0.70 [-0.94, 2.34]	0.94b

sd= standard deviation
CI= confidence interval
a: adjusted for BMI, sports participation and gender
b: adjusted for BMI and sports participation



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AUTOPHAGY AND OSTEOARTHRITIS DEVELOPMENT

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Purpose: We studied here macro-autophagy (thereafter referred as to autophagy), a lysosomal recycling process. Lysosomal protein recycling processes promote cell survival during nutrition depletion by recycling cellular building blocks. Autophagic activity modulation during osteoarthritis progression had been pointed out. However, there is no study directly studying the role of autophagy in osteoarthritis progression.
Methods: To functionally address the role of autophagy in vivo, we generated mice with cartilage specific ablation of Atg5 gene, which is indispensable for autophagy. We analysed the knee joints of these mice at 2, 6 and 12 months of age.
Results: At 2 and 6 months, no differences in bone length or bone and cartilage shape were observed between transgenic animals and wild type in both strains. However, at the age of 1 year, Atg5cKO joints showed features of OA whereas wild type animals had healthy joints (OARSI score: WT: 0,3±0,19; Atg5cKO: 12,66±3,59;N=5; p=0,007). The analysis of 2 months old Atg5cKO knees have higher cell death level (TUNEL, WT: 5,2±3,13; Atg5cKO: 17,03±4,06; N=6; p=0,024). This was correlated with an increase in caspase 3 (WT: 1,88±0,82; Atg5cKO: 8,45±2,3; N=5; p=0,031) and caspase 9 (WT: 0,72±0,39; Atg5cKO: 3,58±1,09; N=5; p=0,043) positive cells.
Conclusions: Our results suggest that autophagy protects chondrocytes from caspase mediated apoptosis and inhibition of this process leads to chondrocyte apoptosis and tissue breakdown.

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CONTRIBUTION OF ELF3 TO CARTILAGE DAMAGE IN A NON-INVASIVE MECHANICAL LOADING MOUSE MODEL WITH OSTEOARTHRITIS-LIKE PATHOLOGY

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Purpose: The E74-like Factor 3 (Elf3) has been shown to contribute to cartilage degradation and mediate inflammatory responses in chondrocytes via up-regulation of downstream targets genes, including Mmp13. In this study, we aimed to assess whether cartilage-specific Elf3 deficiency protects against to, or attenuates the progression of cartilage degradation in vivo, using an a non-invasive mechanical loading mouse model that triggers OA-like pathology.
Methods: We generated Col2a1CRE-driven cartilage-specific Elf3-knockout (Col2Cre:Elf3f/f) mice, that showed no obvious differences in size, weight and growth plate morphology when compared to wild-type counterparts. We subjected the left knees of 12-weeks-old male Col2Cre:Elf3f/f (KO) and Elf3f/f Cre-negative controls (WT) to 1 and 4 weeks of non-invasive mechanical loading, at 9N for 1200 cycles/day. The right knees were used as non-loaded controls. At 1-week post-loading, we isolated knee articular cartilages from three KO or three WT mice, and the loaded and control cartilages from the KO or WT littermates were pooled together for the isolation of total RNAs and subsequent RTqPCR analyses. At 4-weeks post-loading, knees were harvested followed by fixation and decalcification in paraformaldehyde and EDTA, respectively. Histological assessment of OA was conducted on Safranin-O/fast green stained serial coronal sections, following the OARSI recommendations for assessment of OA in the mouse.
Results: Our initial RTqPCR analyses showed increased Mmp13 and Ptgs2 expression in the loaded legs of WT animals compared to unloaded controls. Interestingly, the expression of these genes was down-regulated in KO animals, highlighting the role of Elf3 in controlling these downstream effectors. Preliminary histological analyses of WT (n=3) and KO (n=3) knee joints at 4-weeks post-loading indicated a trend towards reduced cartilage degradation scores in the KO animals, which is in agreement with the results obtained by RTqPCR and with the notion that Elf3 contributes to cartilage degradative processes.
Conclusions: We here provide preliminary evidence that Elf3 contributes to cartilage degradation in vivo, using a non-invasive mechanical loading mouse model. Cartilage-specific Elf3 KO animals show a trend towards reduced cartilage erosion following loading, which correlated

with the reduced Mmp13 and Ptgs2 mRNA detected in the KO animals. The contribution of Elf3 to load-induced cartilage damage and the identification of targets and mechanism of action in vivo merits further investigation.

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INHIBITION OF CTX-II RELEASE BY CATHEPSIN K INHIBITION IN VIVO BUT NOT IN VITRO SUGGESTS THAT ANTI-RESORPTIVE THERAPY PROTECTS CARTILAGE

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Purpose: Loss of subchondral bone precedes both reduced joint space in human OA and cartilage loss in animal models. This suggests that resorption of subchondral bone might cause or aggravate cartilage loss. Consistent with this, anti-resorptives given *in vivo* suppress release of CTX-II, a peptide fragment generated from cleavage of type II collagen, in several species and prevent disease progression in animal models and in humans. Thus, loss of subchondral bone may destabilise cartilage and induce its degradation by chondrocytes. However, an alternative explanation for suppression of CTX-II has recently been proposed i.e. that osteoclasts themselves release CTX-II when they resorb calcified cartilage, so that there is less release when resorption is inhibited. We therefore tested whether osteoclasts generate CTX-II from cartilage and if this was done in a MMP- or cathepsin K-dependent manner. We used osteoclast-induced CTX-I release from subchondral bone as a comparator. We also compared the *in vitro* data with the effect of a cathepsin K inhibitor *in vivo* on urine CTX-I and CTX-II levels in female beagle dogs subjected to partial medial meniscectomy, an experimental model of OA[1].
Methods: For the *in vitro* study, human osteoclasts were incubated on slices of subchondral bone and overlying calcified and hyaline cartilage ('bone-cartilage') taken from patients with OA. CTX-I and CTX-II levels were measured after a first incubation period for 24 h to establish a baseline release of the two respective biomarkers. The cultures were then incubated with the selective cathepsin K inhibitor MV061194 (300 nM, n=4), or the broad spectrum MMP-inhibitor GM6001 (10 μM, n=4), or vehicle (n=4) for another 24 h. Results were expressed as CTX-I or CTX-II release compared to baseline. In a previously performed experimental dog OA study, female beagle dogs were subjected to partial medial meniscectomy and treated with the selective cathepsin K inhibitor MIV-711 (30 μmol/kg p.o., n=15) or vehicle (p.o., n=15) for 28 days starting the day before surgery. Urine was collected for assessment of CTX-I and CTX-II levels and creatinine. All data are expressed as mean ± SEM.
Results: Osteoclast incubation on the bone-cartilage slices increased CTX-I levels from <LLQ (0.44 nM) to 155 ± 24 nM. CTX-II levels from the same slices were increased from 34 ± 6 pg/ml to 533 ± 23 pg/ml. As expected, the cathepsin K inhibitor MV061194 strongly suppressed CTX-I release (vehicle controls: 76±13% compared to baseline; MV061194: 26±4%). However, there was no change in CTX-II release in presence of the cathepsin K inhibitor (vehicle controls: 92±5% compared to baseline; MV061194: 88±8%). In contrast, the MMP inhibitor GM6001 reduced CTX-II to 45±5% of baseline release. These data however do not exclude that osteoclasts could release other cleavage fragments from type II collagen in a cathepsin K-dependent manner. In female beagle dogs, after 28-day treatment, the selective cathepsin K inhibitor MIV-711 reduced urinary CTX-I levels to 14±1% compared to baseline and CTX-II levels to 20±2%.
Conclusions: Osteoclasts release CTX-I and CTX-II from bone/cartilage explants *in vitro*. The CTX-I release *in vitro* is cathepsin K-dependent while the CTX-II release is not. By contrast, selective cathepsin K inhibition reduces urinary levels of CTX-I and CTX-II in a similar fashion in dog. In dog, these reductions translated into structural benefit on cartilage[1]. Thus, this provides evidence that the reduction in CTX-II *in vivo* by cathepsin K inhibition is probably not caused by direct suppression of osteoclast-evoked CTX-II release. Rather, direct inhibition of cathepsin K-mediated bone resorption protects cartilage, and thereby indirectly reduces the release of CTX-II.
Reference
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KNEE KINEMATIC DIFFERENCES IN ANTERIOR CRUCIATE LIGAMENT DEFICIENT SUBJECTS PRIOR TO RECONSTRUCTION IS ASSOCIATED WITH KNEE T1ρ CARTILAGE RELAXATION TIME AT LONGITUDINAL FOLLOW-UP

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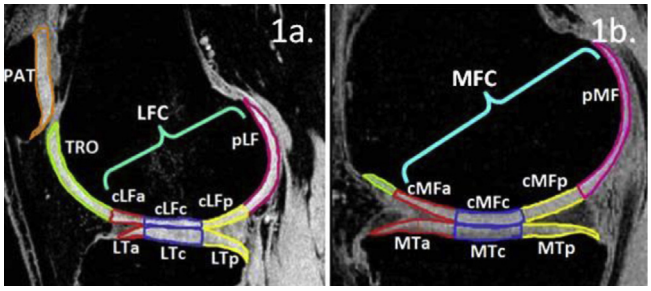
Purpose: This study aimed to identify possible biomechanical markers in gait alterations in anterior cruciate ligament (ACL)-deficient knees prior to reconstruction to predict T1ρ relaxation times.
Methods: Subjects: Motion analysis data were recorded at baseline (n=49, female:male = 20:29, age: 29.63±8.63 years, BMI: 23.8±2.66 kg/m^2) after injury and before ACL reconstruction. Gait Analysis: At baseline, three-dimensional motion analysis was captured while subjects walked at a controlled speed (1.33±0.6667 m/s). Lower-extremity kinematics data were recorded using AMTI force plate (Watertown, MA, USA, sampling frequency = 1000Hz) and Vicon motion capture system (Oxford Metrics, UK, sampling frequency = 250 Hz). Joint external moments and impulses were then computed using Visual3D Software (C-Motion, Germantown, MD, USA). MR Protocol: Of the 49 subjects that conducted motion analysis at baseline, 39 patients returned 12 months following surgery to conduct MR imaging of both knees using a 3 Tesla scanner (GE healthcare) and an 8-channel knee coil (Invivo). Comprehensive cartilage T1ρ relaxation times were calculated using previously validated and published methods by our group. Cartilage was divided into subcompartments to examine regional variations in cartilage health (Figure 1). Mean T1ρ relaxation times were calculated for each global compartment and its corresponding subcompartments.



Statistic Analysis: Pearson product correlation coefficients were calculated between T1ρ relaxation times at the 12-month timepoint and gait characteristics obtained at baseline prior to surgery Variables historically in literature that could be associated with knee osteoarthritis were examined, such as peak knee extension adduction moments and impulses, and hip flexion moments.
Results: In injury knees, baseline lower peak hip flexion moment, lower peak knee extension moment in the 1st half of stance phase and lower peak knee adduction moment in the 2nd half of stance phase were significantly correlated with increased cartilage T1p at 12-months. (Table 1)

Table 1: Baseline motion analysis correlated to T1p at 12-months after ACL reconstruction in injured knees.

Injured Knee at Baseline[c]	T1p[a]
Peak Hip Moment Flexion	cLT: 0.0446 (0.323)[b] TRO: 0.0314 (0.345) cLF.p: 0.0257 (0.334)
Peak Knee Moment Extension (1st half of Stance Phase)	LFC: 0.0383 (-0.333) PAT: 0.00066 (-0.448) TRO: 0.0309 (-0.346) cMF.p: 0.0463 (-0.321)
Peak Knee Moment Addduction (2nd Half of Stance Phase)	cLF.p: 0.0376 (-0.331)

[a] Injured knee 1 year after reconstruction
[b] P-value (R)
[c] External moments